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Two-Dimensional Crystallization of Membrane Proteins: Screening Strategies

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Membrane proteins play fundamental roles in a broad range of biological processes and represent more than half of the current drugs targets [1]. Electron microscopy has been effective in producing high resolution representations, but the low signal-to-noise ratio inherent to the imaging process requires averaging of thousands of perfectly aligned proteins. Two-dimensional (2D) crystallization constitutes an advantage over single particle analysis, as the proteins are already aligned within the crystal. Furthermore, as opposed to X-ray analysis of three-dimensional crystals, the proteins are inserted into a lipid bilayer which makes the reconstitution closer to the native environment.

For 2D crystallization, proteins and lipids solubilized in detergents are mixed at defined lipid-to-protein ratios (LPRs). As the detergent is removed, usually by dialysis, optimal conditions will produce crystalline sheets or helical tubes. Finding those optimal conditions is however a difficult task as many factors need to be considered: the lipid species, the detergent species used for solubilization, the LPR, the buffer composition (including pH, salts and protein ligands), the temperature, and the rate of detergent removal. When a new protein enters a 2D crystallization pipeline, it is not obvious where to start the screening process. Although high throughput tools have been developed in recent years [2], screening the entire space of conditions is logistically impossible. We have therefore developed different screening strategies which will be presented here. These strategies offer alternatives to the systematic screening approach that varies only one or two factors at a time. Our first two strategies are biased toward previous successful 2D or 3D crystallization experiments. The third strategy is an incomplete factorial which covers a wide range of crystallization parameters conditions in an unbiased way.

Our first screen is reserved for proteins that have already produced 3D crystals. In this case, we design a custom screening matrix centered on those conditions. In particular, the relevant pH, salts and protein ligands are adopted while screening a variety of lipids at different LPRs.

Our second screen is a sparse matrix of conditions biased toward successful 2D crystallization experiments reported in the literature. To create this matrix, we combined the data from 94 successful crystallization conditions applied to 57 unique proteins. The parameters include pH, monovalent and divalent salt concentrations, temperature and lipids and a specific set of conditions was chosen based on a k-means algorithm. The resulting matrix comprises 10 conditions, a number of different lipid species and 3 to 6 LPRs, depending on the amount of available protein. To allow the community to access this screening matrix and to create new matrices based on the input data, the database has been made available through the Sesame LIMS, which can be accessed through the World Wide Web: www.temimps.nysbc.org/TwoDCrystallizationDatabase.html.

Our third screen is based on an incomplete factorial matrix which has been designed to sample the primary factors influencing the crystallization process: 8 lipid mixtures, 3 lipid co-factors, 5 detergents for lipid solubilization, 8 LPRs (0.1 to 1.5), 10 pHs (4.5 to 9), 4 monovalent salts, 2 divalent salts, 3 different salt concentrations, 5 temperatures, and 4 ways to control the detergent removal rate. Based on the design of experiment methodologies proposed by Fischer [3] and the similar approach developed for 3D crystallization [4], our matrix results in 90 unique conditions. The selection process assured that the conditions were assigned randomly and that the first-order interactions between the different factors remain balanced.

The first approach based on 3D crystallization experiments lead to 2 new crystal forms out of 7 proteins tested. Out of the 9 proteins tested with the sparse matrix, 5 have given crystals. Finally, crystals of 4 out of 6 proteins have been obtained with the incomplete factorial screen. The research is conducted under the TEMIMPS research consortium (Transcontinental EM Initiative for Membrane Protein Structure) [5].

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